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13. ABSTRACT (Maximum 200 Words)

The conserved oligomeric Golgi (COG) complex was identified as one of the evolutionary conserved protein complexes that regulate a cis-Golgi step in intracellular vesicular transport. This evolutionary conserved complex is composed of eight subunits. Mutations in the COG complex subunits result in defects in basic Golgi functions: glycosylation of secretory proteins, protein sorting and retention of Golgi resident proteins. We propose that the COG3 protein plays one of the main roles in these processes. We utilized RNA interference assay to knockdown COG3p in HeLa cells to determine the effect of its depletion on Golgi proteins localization. siRNA dependent Cog3 depletion causes rapid Golgi fragmentation and possibly accumulation of Golgi resident proteins in transport vesicles. Furthermore in COG3 depleted cells level of COG1, 2, 4 and 8 is also reduced while the level of COG5 and 6 subunits is not changed. We found that the COG complex physically interacts with components of intra-Golgi trafficking machinery including v-SNARE GS28. COG3 protein is localized on Golgi in normal conditions but in breast cancer cells in addition to the Golgi it is also found on peripheral structures where it is colocalized with SNARE protein GS28. We concluded that mammalian COG complex serves as a "docking station" for retrograde intra-Golgi vesicles and that the lobe A of COG complex (subunits 1-4) is essential for this process. These results help to further define the COG complex function in protein trafficking.

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Introduction

In breast, colon and skin cancers, the unusual production and secretion of aberrantly glycosylated proteins and lipids on the surface are associated with disease progression, metastasis and poor clinical outcome (1). Glycosylation abnormalities concern both N-linked and O-linked carbohydrate chains on glycoproteins and glycolipids (2). They likely impair many basic cellular functions, since terminal oligosaccharide units serve as highly specific biological recognition molecules implicated in major regulatory processes of the cell. These phenotypic changes in malignant cells highly correlate with marked structural and functional disorganization of the Golgi apparatus (2).

The Conserved Oligomeric Golgi (COG) complex is a peripheral membrane protein complex localized on cis/medial Golgi cistern. This evolutionary conserved complex is composed of eight subunits that are thought to be located in two lobes, the first lobe A containing the COGs 1-4 and the second lobe B the COGs 5-8 (3). Mutations in the COG complex subunits result in defects in basic Golgi functions: glycosylation of secretory proteins, protein sorting and retention of Golgi resident proteins. Cog1 and Cog2 deficient CHO cells are viable, but exhibit defects in multiple Golgi glycosylation pathways establishing a role for the COG complex in mammalian Golgi function (3, 4). Recently, two siblings were described with a fatal form of congenital disorders of glycosylation (CDG) caused by a mutation in the gene encoding COG7. The mutation impairs integrity of the of the COG complex and alters Golgi trafficking, resulting in disruption of multiple glycosylation pathways (5). All these data indicate that COG complex may participate in Golgi protein trafficking.

Body

During the first year of traineeship period the focus of my research was directed towards the study of the localization of COG complex and Golgi proteins in tumor cells. In order to complete this project, I obtained new training, especially in the area of fluorescent microscopy and the RNA interference technique. Now I have excellent approach for study of the role of the COG complex in the abnormalities of glycoprotein processing in breast cancer cells. For this study the HeLa cells and MCF7 cells lines were obtained from ATCC (American Type Culture Collection, Rockville, MD); human breast cancer (HBC) cells SUM 52PE, 159PT, 185PE, 229PE and 1315MO2 (6) were kindly provided from Dr. Steve Ethier's laboratory (University of Michigan, http://www.cancer.med.umich.edu/breast_cell/Production) and normal breast cells (HB2) line was kindly provided from Dr. Kurten (University of Arkansas for Medical Sciences, Arkansas).

1. COG3 protein in normal conditions is localized on Golgi. In breast cancer cells it is also localized on peripheral structures.

COG complex localization in cancer cells probably reflect mislocalization of its membrane receptor(s) (Fig. 1).

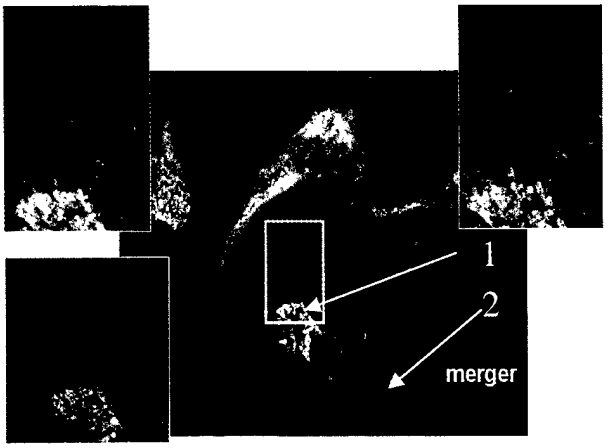

Fig. 1. Localization of COG3p in HBC SUM1315MO2. Immunofluorescence was revealed that COG complex localized both on Golgi structure (1) and on peripheral structures (2). I discovered that GS28 is colocalized with COG3 on peripheral structures in breast cancer SUM1315MO2 cells.

2. Depletion of COG3 by siRNA interference on Golgi proteins localizations

I utilized RNA interference assay to knockdown COG3. Three different siRNA have been used. Only one of them was active in Hela cells. The left panel (Fig. 2) shows control cells, transfected only with reagent. On the right panel are cells after 72 h of siRNA transfection. The quantity of COG3p was dramatically reduced in siRNA transfected cells.

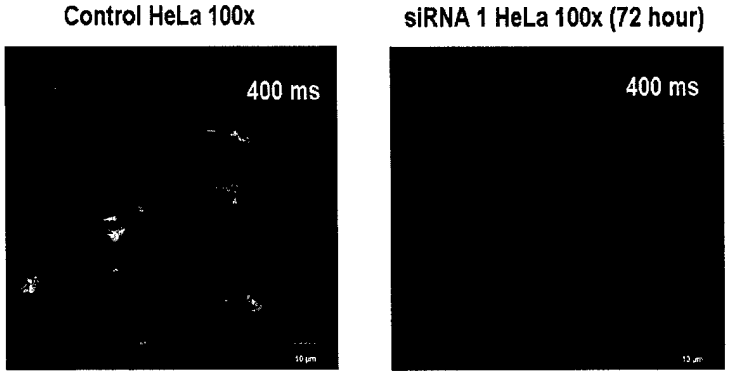

Fig. 2. siRNA COG3 transfection induces degradation of COG3p. Immunofluorescence localization of COG3p in HeLa cells after transfected by siRNA COG3.

Depletion of COG3 by siRNA induces reduction of COG1,2,4,8 subunits (Fig. 3) and GS28 without influence on COG5,6 subunits in HeLa cells.

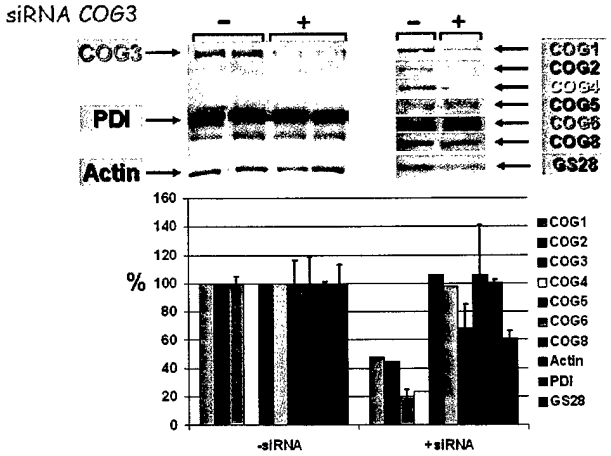

Fig. 3. Depletion of COG3p changes stability of COG complex and v-SNARE GS28.
Western blot shows that depletion of COG3 by siRNA induces reduction of COG1,2,4,8 subunits and GS28 without influence on COG5,6 subunits in HeLa cells. siRNA transfection didn't change concentration of the control proteins Protein Disulphide-Isomerase (PDI) and Actin.

3. The interaction of COG3 protein with v-SNARE GS28 *in vivo.*

We performed immunoprecipitation of COG3 containing complexes from CHAPS solubilized Rat liver Golgi membranes (Fig. 4).

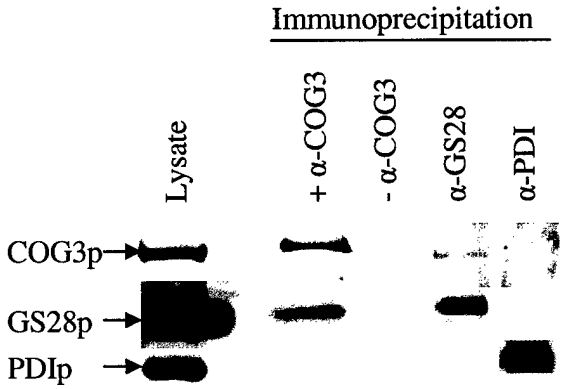

Fig. 4. Co-immunoprecipitation of COG3 protein with v-SNARE GS28 from rat liver Golgi membranes. The liver Golgi membranes were solubilized in 2 % CHAPS, 40 mM TRIS pH 7.4, 300 mM NaCl, protease inhibitor cocktail. Immunoprecipitation of protein were carry out with 2 μg of antibodies. 20% of lysate was loaded as control.

4. Redistribution of the Golgi proteins in different cell compartments after siRNA COG3. COG3 depletion is accompanied by reduction in COG1, 2 and 4 protein levels and by rapid accumulation of intra-Golgi recycling vesicles that carry v-SNARE GS28 and cis-Golgi glycoprotein GPP130 (Fig.5 and Fig. 6).

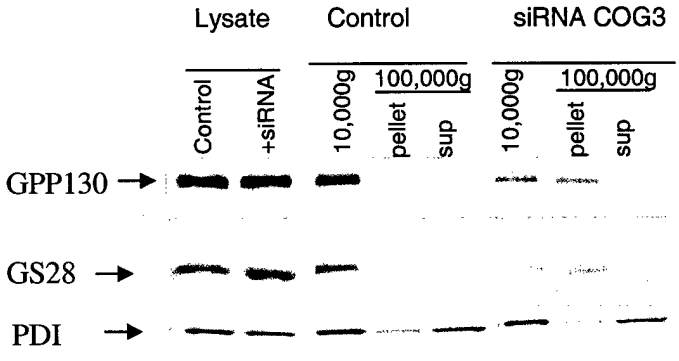

Fig. 5. Mislocalization of GPP130 and GS28 proteins in HeLa cells after siRNA COG3 treatment.
After 72 h of transfection by siRNA HeLa cells were scraped from 60-mm dishes in 0.3 ml of 20 mM Hepes-KOH buffer, pH 7.4, supplemented with a proteinase inhibitor mixture, disrupted using a Potter homogenizer and separated on several fraction by centrifugation (Sup. – supernatant).

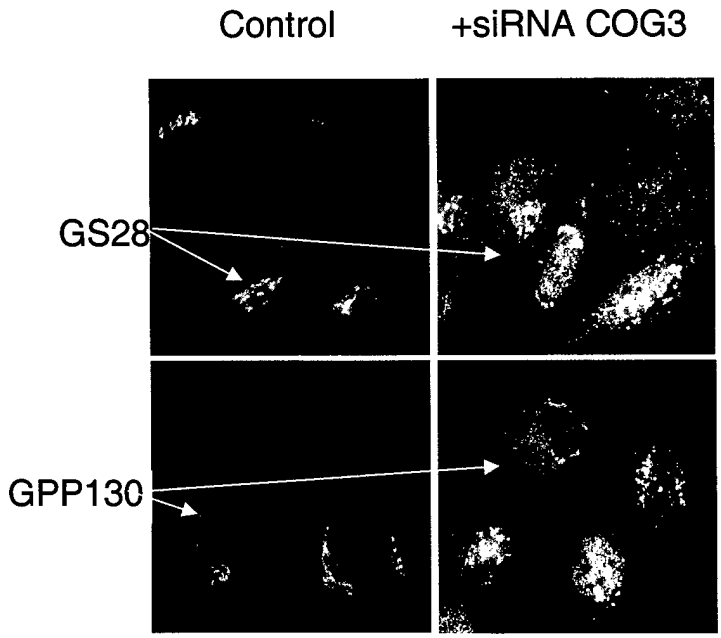

Fig. 6. siRNA dependent Cog3 depletion cause Golgi fragmentation and possible accumulation of Golgi resident proteins in transport vesicles. In siRNA transfected HeLa cells Golgi protein GPP130 and v-SNARE GS28 are redistributed from perinuclear region, 48 h siRNA transfetion.

Key research accomplishments

1. I concluded that mammalian COG complex serves as a "docking station" for retrograde intra-Golgi vesicles and that the lobe A of COG complex (subunits 1-4) is essential for this process.

Reportable outcomes

1. Participation in the "Student Research Week" of the College of Medicine, University of Arkansas for Medical Sciences, Arkansas on April 19 – 22, 2004,

Conclusions

1. For the first time I have demonstrated that acute depletion of COG complex activity resulted in inhibition of tethering of intra-Golgi vesicles.
2. In addition, Golgi-located COG complex physically interacts with GS28 and co-localizes with GS28 on peripheral membrane structures in SUM1315MO2 breast cancer cells.
3. I concluded that mammalian COG complex serves as a "docking station" for retrograde intra-Golgi vesicles and that the lobe A of COG complex (subunits 1-4) is essential for this process.

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Appendices

1.

UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES
COLLEGE OF MEDICINE
STUDENT RESEARCH WEEK
April 19 – 22, 2004
ABSTRACT FORM

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CATEGORY: GRADUATE STUDENT:___ MEDICAL STUDENT:___ HOUSESTAFF:___ POSTDOC FELLOWS: X

On Wednesday, April 21st presenters must man their poster to be eligible for an award
Mark a time slot best for you 9:00-11:00 AM X 1:00-3:00 PM___

siRNA dependent Cog3 depletion causes rapid Golgi fragmentation.

Sergey N. Zolov, Department of Physiology and Biophysics UAMS

The conserved oligomeric Golgi (COG) complex was identified as one of the evolutionary conserved protein complexes that regulate a cis-Golgi step in intracellular vesicular transport. This evolutionary conserved complex is composed of eight subunits. Mutations in the COG complex subunits result in defects in basic Golgi functions: glycosylation of secretory proteins, protein sorting and retention of Golgi resident proteins. We propose that the COG3 protein plays one of the main roles in these processes. We utilized RNA interference assay to knockdown of COG3p in HeLa cells to determine the effect of its depletion on Golgi proteins localization.

siRNA dependent Cog3 depletion cause rapid Golgi fragmentation and possibly accumulation of Golgi resident proteins in transport vesicles. Furthermore in COG3 depleted cells level of COG1, 2, 4 and 8 is also reduced while the level of COG5 and 6 subunits is not changed. We found that the COG complex physically interacts with components of intra-Golgi trafficking machinery including SNAREs and vesicle tether GM130. COG3 protein in normal conditions is localized on Golgi but in breast cancer cells in addition to the Golgi it is also found on peripheral structures where it colocalized with SNARE protein GS28. These results helps to further define the COG complex function in protein trafficking.

The work presented is substantially that of the presenter and hence is eligible for award consideration:

Zolov
Student Signature

Faculty Sponsor Signature

Department: Physiology # 505

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